

Formulation and evaluation of sardine pickles in oil: a study on sensory attributes, proximate analysis, and microbial content

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Abstract

In the production of sardine pickles in oil using spotted sardinella (*Amblygaster sirm*) as the main ingredients, four formulations were prepared, incorporating palm oil and vinegar as primary ingredients. These formulations underwent sensory evaluation tests with 50 panellists, assessing attributes including colour, odour, texture, taste, aftertaste, and overall acceptance. Proximate analysis and microbial tests were also conducted to assess nutritional composition and microbial safety. The sensory evaluation identified the F4 sample, which had the highest palm oil (48.1%) and the lowest vinegar (2.5%) contents, as the optimal formulation, garnering the highest mean score compared to other formulations. This preferred sample exhibited moisture (48.66±3.04%), ash (0.36±0.21%), fat (9.03±5.18%), and protein (41.95±0.52%) content with zero carbohydrates. Microbial tests revealed a decline in microbial activity over the observation period, attributed to the acidic condition of the sample, rendering microorganisms such as *Escherichia coli*, yeasts, and mould unable to thrive. Overall, the production of sardine pickles in oil with the optimised formulation proved successful, achieving the research objectives.

1. Introduction

Fish, including sardines, stands as a vital source of essential nutrients crucial for daily nutrition (Tavares *et al.*, 2021). Packed with essential nutrients, including amino acids, fat-soluble vitamins, and micronutrients, and is high in unsaturated fatty acids. Fish, like chicken and meat, is a key protein source for Malaysians due to its rich nutritional profile and high protein content. Sardines, belonging to the Clupeidae family, are indigenous to diverse regions and can vary in size from 15 cm to 29 cm, contingent on their species (Rajan, 2018). In Malaysia, several sardine species thrive, including spotted sardinella (*Amblygaster sirm*), blacksaddle herring (*Herklotsichthys dispilonotus*), smoothbelly sardinella (*Amblygaster sirm*), white sardinella (*Sardinella albella*), and deepbody sardinella (*Sardinella brachysoma*). These species are known to

prey on phytoplankton or microalgae and are commonly found in coastal waters, lagoons, and regions of the Central West Pacific and Indo-West Pacific according to FishBase (Froese and Pauly, 2023). Malaysian fishermen typically supply fresh fish to the market, but the perishable nature of sardines necessitates measures to ensure food safety (Tavares *et al.*, 2021). To tackle this challenge, processing sardines for canned consumption extends shelf life and enhances consumer value.

Preserving food quality is a critical concern within the food industry, and it hinges on factors like storage temperature, relative humidity, and gas composition (Mercier *et al.*, 2019). Additionally, potential microbial contamination poses a significant risk (Sevindik, 2018), especially for perishable items, which have a limited shelf life and can become unsafe if compromised

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(Mercier *et al.*, 2019). To address this, various food preservation techniques are employed in the food and beverage industry.

Food preservation is an ancient practice aimed at prolonging the shelf life of perishables while retaining their nutritional value. It encompasses two main categories: conventional and modern techniques. Commonly used preservation methods include drying, freezing, refrigeration, pasteurisation, chemical additives (Amit *et al.*, 2017), thermal processing, and pickling (Sharif *et al.*, 2017). Among these, pickling is recognised as one of the oldest known preservation methods (Behera *et al.*, 2020). This technique relies on salt or vinegar as primary agents in the preservation process. Thus, this study aimed to formulate oil-based sardine pickles, guided by sensory evaluation, proximate analysis, and microbial testing.

2. Materials and methods

2.1 Preparation of raw materials

To ensure the high quality and freshness of the fish used in this product, spotted sardinella were procured directly from fishermen or trusted suppliers of fresh seafood at the Pasar Besar, Kota Kinabalu, Sabah, Malaysia. The selected fish were chosen for their size to maximise the quantity of fish content and were stored in a cool box filled with rock water to maintain their freshness and temperature. Additionally, other ingredients such as palm oil, garlic, vinegar, salt, chilli powder, black pepper seeds, and rosemary leaves were sourced directly from reputable suppliers of raw materials in the vicinity of Kota Kinabalu. These non-fish ingredients were stored under proper conditions to prevent any potential contamination or deterioration in quality.

2.2 Formulation of pickle

For the production of sardine pickles in oil, a control formulation, as outlined by Rahman *et al.* (2019), was employed as a reference with minor adjustments. The formulation has been modified and tailored to suit the

specifics of the study at hand. Table 1 provides a detailed breakdown of the formulation intended for use.

2.3 Fish pickling process

The sardines were thoroughly washed with clean water to ensure the removal of any dirt or unwanted residue. The fish's head and tail were then trimmed and discarded. The cleaned fish were immersed in a salt solution with a ratio of 30.0 g of salt to 120.0 mL of water (a 1:4 ratio) and left to soak for 30 mins. Subsequently, the fish were fried until fully cooked. Once fried, the fish were allowed to cool at room temperature before being placed in a sterilised glass container along with 10.0 g of briefly fried garlic, 2.5 g of black pepper seeds, 2.0 g of chilli powder, and 0.5 g of rosemary leaves. The container was then filled with oil and vinegar according to the provided formulation. Finally, the bottle was tightly sealed, cleaned to remove any oil residue, labelled, and stored in a dry area at room temperature for further analysis.

2.4 Sensory evaluation

A total of 50 untrained panellists, including undergraduates and staff from Universiti Malaysia Sabah, Kota Kinabalu, were involved in the sensory evaluation. The sensory evaluation took place at the Sensory Evaluation Laboratory, Faculty of Food Science and Nutrition. The objective of this evaluation was to assess the level of acceptance of the formulated sardine pickles in oil and to determine the formulation that received the highest score from the panellists. During this evaluation, various sensory attributes, including colour, odour, texture, taste, aftertaste, and overall acceptance, were assessed. The panellists rated each attribute using the hedonic scale: very good (7), good (6–5), medium (4–3), and bad (2–1).

2.5 Proximate analysis

Moisture content of the sardine pickles in oil was determined using the Association of Official Analytical Collaboration (AOAC) International Official Method

Table 1. Five formulations of raw materials for sardine pickles in oil.

Raw material	Formulation				
	Control	F1	F2	F3	F4
Sardine	150.0 g	150.0 g	150.0 g	150.0 g	150.0 g
Palm oil	0.0 mL	130.0 mL	150.0 mL	170.0 mL	190.0 mL
Vinegar	200.0 mL	70.0 mL	50.0 mL	30.0 mL	10.0 mL
Salt	30.0 g	30.0 g	30.0 g	30.0 g	30.0 g
Garlic	10.0 g	10.0 g	10.0 g	10.0 g	10.0 g
Black pepper	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
Chili powder	2.0 g	2.0 g	2.0 g	2.0 g	2.0 g
Rosemary leave	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g

950.46, by oven-drying approximately 2.0 g of sardine pickle samples at 103°C overnight. Ash content was determined using AOAC International Official Method 938.08, by incinerating approximately 2.0 g of samples in a furnace overnight at 550°C until a constant weight was achieved. Fat content was determined using AOAC International Official Method 960.39, by solvent extraction of approximately 2.0 g of samples. Crude protein content was determined by the Kjeldahl method using AOAC International Official Method 981.10, with approximately 0.5 g of sample digested and analysed using a Kjeltex analyser. The carbohydrate content of samples was determined by difference, according to AOAC International Official Method 986.25.

2.6 Microbial test

To detect microorganisms, the pour plate technique was carried out following the methods of Atanassova *et al.* (2014) and Zheng *et al.* (2005). Briefly, 10.0 g of sardine pickle samples were homogenised with 90.0 mL of sterile buffered peptone water, followed by serial tenfold dilutions (10^{-1} to 10^{-7}). For total plate count (TPC), 1 mL of each appropriate dilution was plated on Plate Count Agar (PCA) and incubated at 37°C for 48 hrs to assess total aerobic mesophilic bacteria. *Escherichia coli* was enumerated on eosin-methylene blue agar (EMBA), incubated at 37°C for 48 hrs. Yeast and mould were cultured on Potato Dextrose Agar (PDA) at 25°C for 7 days. Results were expressed as colony-forming units per millilitre (CFU/mL).

2.7 Statistical analysis

All data are presented as mean \pm standard deviation (SD) from three separate trials. The sensory evaluation results were analysed using a one-way analysis of variance (ANOVA) test. If a significant difference was detected in the ANOVA, Tukey's Honestly Significant Difference (HSD) test was subsequently applied in the sensory evaluation. Meanwhile, the data obtained from the proximate analysis were analysed using the independent-samples t-test to compare mean values between groups. This statistical analysis was performed

using IBM SPSS Statistics 28.

3. Results and discussion

3.1 Sensory evaluation results

Following the stated formulations, sensory tests were conducted with 50 panellists assessing attributes such as colour, odour, texture, taste, aftertaste, and overall acceptance. Scores were assigned based on a hedonic scale. The summarised results are shown in Table 2.

3.1.1 Colour

The colour evaluation ranged from 4 to 5, indicating a neutral to slightly favourable preference. The F3 sample, with a mean score of 5.14 ± 1.07 , was the preferred choice for colour. Evaluation of colour attributes across all formulations revealed no significant differences ($p > 0.05$). This uniformity may be attributed to consistent ingredient proportions, with variations mainly in oil and vinegar content. Additionally, the inclusion of chilli powder contributed to a consistent colour profile. However, this uniformity resulted in a less visually appealing outcome, leading to a lower mean acceptance score.

3.1.2 Odour

In terms of odour, mean scores ranged from 4 to 5, indicating a neutral to slightly favourable preference. The F4 sample was the preferred choice for this attribute, scoring 5.30 ± 1.40 . This preference is likely due to the lower vinegar content in the F4 sample compared to the other samples. According to Bartowsky and Henschke (2008), the aroma of this vinegar is influenced by acetic acid, which impacts olfactory perception. Significant differences were observed for the F4 sample ($p < 0.05$) when compared to the F1 sample, while no significant differences ($p > 0.05$) were noted between the control, F2, and F3 samples. The difference in odour perception between the F1 and F4 samples can be attributed to their varying vinegar content, with the F1 sample containing over 17%, compared to the F4 sample, which uses less than 3%.

Table 2. Sensory evaluation results using the hedonic scale for five sardine pickle formulations in oil.

Attribute	Formulation				
	Control	F1	F2	F3	F4
Colour	4.92 \pm 1.51 ^a	4.94 \pm 1.20 ^a	4.88 \pm 1.27 ^a	5.14 \pm 1.07 ^a	4.88 \pm 1.33 ^a
Odour	4.76 \pm 1.47 ^{ab}	4.24 \pm 1.71 ^b	4.72 \pm 1.42 ^{ab}	4.96 \pm 1.47 ^{ab}	5.30 \pm 1.40 ^a
Texture	4.70 \pm 1.27 ^a	4.62 \pm 1.41 ^a	4.52 \pm 1.30 ^a	4.86 \pm 1.37 ^a	5.18 \pm 1.30 ^a
Taste	4.16 \pm 1.02 ^{ab}	3.66 \pm 1.80 ^b	4.36 \pm 1.66 ^{ab}	4.54 \pm 1.52 ^a	4.74 \pm 1.63 ^a
Aftertaste	4.42 \pm 1.37 ^{ab}	3.62 \pm 1.60 ^b	4.34 \pm 1.70 ^{ab}	4.38 \pm 1.47 ^{ab}	4.62 \pm 1.65 ^a
Overall acceptance	4.22 \pm 0.98 ^{bc}	3.98 \pm 1.53 ^c	4.76 \pm 1.33 ^{ab}	4.72 \pm 1.46 ^{abc}	5.00 \pm 1.50 ^a

Values are presented as mean \pm SD of triplicate. Values with different superscripts in the same column are statistically significantly different by one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

3.1.3 Texture

The texture scores ranged from 4 to 5, with the highest mean score of 5.18 ± 1.30 recorded for the F4 sample. This indicates that it was the preferred choice among panellists for texture. However, no significant differences ($p > 0.05$) were observed between the control sample and all the formulations. This may be due to all samples being freshly produced.

3.1.4 Taste

The taste ratings varied between 3 and 4, with the F4 sample achieving the highest average score of 4.74 ± 1.63 , indicating a preference for its balanced flavour among the panellists. Notably, this formulation contained the lowest percentage of vinegar content, totalling 2.5%. The taste of the F4 sample showed no significant differences ($p > 0.05$) compared to all other samples, except for the F1 sample.

3.1.5 Aftertaste

The aftertaste attribute was assessed in the sensory test, with all the sample scores falling between 3 and 4 on the scale, indicating a slight aversion to neutrality. The F4 sample received the highest mean score of 4.62 ± 1.65 , making it the preferred choice among the panellists. The F4 sample also showed a significant difference ($p < 0.05$) compared to the F1 sample, but not compared to the control, F2, or F3 samples.

3.1.6 Overall acceptance

The overall acceptance score reflects the final panel evaluation for sardine pickles in oil. The F4 sample (5.00 ± 1.50) significantly ($p < 0.05$) outperformed the control (4.22 ± 0.98) and F1 (3.98 ± 1.53), but showed no significant difference ($p > 0.05$) compared to the F2 (4.76 ± 1.33) and F3 (4.72 ± 1.46) samples.

3.1.7 Selection of the best formulation

In the sensory evaluation test for sardine pickles in oil, each sample was assessed for attributes such as colour, odour, texture, taste, aftertaste, and overall acceptance. The sample with the highest mean score was considered the best based on these attributes. Taste, odour, and aftertaste were particularly important in determining the best formulation. As shown in Table 2, the F4 sample had the highest mean score, indicating a strong preference by the panel compared to the other samples, although it did not exhibit a significant difference ($p > 0.05$) in comparison based on these attributes. Additionally, the F4 sample also received the highest mean score for overall acceptance. Therefore, the F4 sample was selected as the optimal formulation for sardine pickles in oil production and underwent further

assessment of its proximate composition and microbial content.

3.2 Proximate composition

For proximate composition analysis, the F4 sample, representing the best formulation from the sensory evaluation, was utilised along with the control sample. These samples underwent measurements for moisture, ash, fat, protein, and carbohydrate contents. The results are detailed in Table 3.

Table 3. Proximate composition of control and F4 samples of sardine pickles in oil.

Proximate composition	Percentage (%)	
	Control	F4
Moisture	62.50 ± 2.66	48.66 ± 3.04
Ash	0.16 ± 0.04	0.36 ± 0.21
Fat	4.76 ± 0.65	9.03 ± 5.18
Protein	32.58 ± 1.89	41.95 ± 0.52
Carbohydrate	0.00 ± 0.00	0.00 ± 0.00

Values are presented as mean \pm SD of triplicate. Both groups are statistically significantly different, as determined by an independent samples t-test ($p < 0.05$).

3.2.1 Moisture content

The moisture content analysis showed that the F4 sample had a lower moisture content ($48.66 \pm 3.04\%$) compared to the control ($62.50 \pm 2.66\%$). Rahman *et al.* (2019) reported even higher values ($79.21 \pm 1.43\%$), while Pervin *et al.* (2010) documented a range of 43.85% to 50.89%. Upon closer examination, it became apparent that the F4 sample contained more oil compared to the control samples, which were not immersed in oil. Additionally, the fish underwent a frying process during sample preparation and processing, which could potentially influence the moisture content results in the F4 sample. It is well-documented that the moisture content of fresh fish tends to decrease after frying and cooking (Rahman *et al.*, 2019). Therefore, the utilisation of oils and processing methods such as frying in the preparation of the F4 sample were contributing factors affecting its moisture content.

3.2.2 Ash content

The ash content analysis reveals that the control sample had an ash content of $0.16 \pm 0.04\%$, while the F4 sample had $0.36 \pm 0.21\%$. While the ash content in the F4 sample exceeds that of the control, it remains below the levels reported by Pervin *et al.* (2010) and Rahman *et al.* (2019), at 1.00% and $1.60 \pm 0.24\%$, respectively. Rahman *et al.* (2019) reported that moisture content can affect ash content determination due to moisture loss during frying and cooking. Additionally, Shiriskar *et al.* (2010) suggest

that storage duration may also influence ash content.

3.2.3 Fat content

The fat content analysis shows that the F4 sample had a higher fat content ($9.03 \pm 5.18\%$) than the control sample ($4.76 \pm 0.65\%$). This corresponds with the results from Rahman *et al.* (2019), who reported a fat content of $4.00 \pm 0.45\%$. The increased fat content in the F4 sample is due to the substantial use of palm oil for marination, resulting in sardine pickles in oil. In contrast, the control samples had sardine fish soaked in a vinegar solution after frying. The cooking process with oil also influenced the final fat content analysis results.

3.2.4 Protein content

The control sample exhibited a protein content of $32.58 \pm 1.89\%$, which was lower than that of the F4 sample at $41.95 \pm 0.52\%$. This indicates a discernible difference in protein content between the two. Comparatively, findings from Pervin *et al.* (2010) and Rahman *et al.* (2019) ranged from 13.16–19.13% and $13.17 \pm 0.91\%$, respectively, which were considerably lower. Upon investigation, it was found that the control sample used a higher vinegar content than the F4 sample, causing a shift in pH towards acidity. These pH changes can disrupt the molecular structure of proteins, leading to denaturation. Storage duration also impacts protein content, as denaturation can occur over time. This is consistent with findings by Rahman *et al.* (2019) and Wang *et al.* (2021), where extended exposure to acids was shown to damage protein structure. Consequently, the control sample had lower protein content compared to the F4 sample.

Protein content is also influenced by factors including time, temperature, processing methods, and the age of the fish. Tobin *et al.* (2006) note that older fish tend to have higher fat and protein content compared to younger ones. Elevated temperatures and extended processing times can lead to protein denaturation, affecting nutritional composition (Abraha *et al.*, 2018).

High-temperature frying during sample preparation also contributed to the decrease in protein content. As a result, the protein content of the control sample was lower than that of the F4 sample.

3.2.5 Carbohydrate content

Both the control and F4 samples showed negligible carbohydrate content (0.00 ± 0.00), indicating an absence of carbohydrates in both formulations. This suggests that the pickling process, which primarily involves oil and vinegar, effectively reduces carbohydrate content in the final product (Kim *et al.*, 2016).

3.3 Microbial content

The study employed PCA, EMBA, and PDA techniques for microorganism detection tests over a four week period, and the results are detailed in Table 4. *E. coli* detection tests on the F4 sample indicated no growth throughout the storage period. Similarly, tests for yeast and mould in the first, second, and fourth weeks confirmed their absence, affirming that the F4 sample was free from *E. coli*, yeast, and mould. However, the TPC revealed microbial growth in the F4 sample, starting in the first week at 1.5×10^7 CFU/mL. Subsequent counts were 1.2×10^7 CFU/mL (second week) and 5×10^6 CFU/mL (third week). Interestingly, by the fourth week, microbial growth had ceased, with no detectable colonies observed.

Various factors influence microorganism growth in food. These assessments were vital for establishing the shelf life of the food, especially in the production of sardine pickles in oil. Rahman *et al.* (2019) noted that the presence of bacteria in citrus-based foods tended to be low due to inhibitory factors like salt and food acidity. This finding aligns with the ingredients used in producing the F4 sample of sardine pickles in oil, where salt and vinegar played crucial roles. Additionally, vinegar is acknowledged as an effective acidulant, lowering the pH to levels unsuitable for most microorganisms to thrive (Baygar *et al.*, 2010).

Table 4. Microbial content of TPC, *E. coli*, yeast, and mould from control and F4 samples of sardine pickles in oil.

Duration of storage	Sample	Colony count (CFU/mL)		
		TPC	Yeast and mould	<i>E. coli</i>
Week 1	Control	$3.7 \times 10^7 \pm 0.81$	ND	ND
	F4	$1.5 \times 10^7 \pm 0.24$	ND	ND
Week 2	Control	$1.9 \times 10^7 \pm 0.65$	ND	ND
	F4	$1.2 \times 10^7 \pm 0.21$	ND	ND
Week 3	Control	ND	ND	ND
	F4	$5 \times 10^6 \pm 0.65$	ND	ND
Week 4	Control	ND	ND	ND
	F4	ND	ND	ND

Values are presented as mean \pm SD of triplicate. ND: Not detected.

4. Conclusion

The study identified the F4 sample as the optimal formulation for producing sardine pickles in oil using spotted sardinella. Among five tested formulations, the F4 sample received the highest sensory score, featuring 48.1% oil and 2.5% vinegar content. Although the mean score was modest (5.00), it was most preferred among panellists. Proximate analysis of the F4 sample showed 48.66±3.04% moisture, 0.36±0.21% ash, 9.03±5.18% fat, and 41.95±0.52% protein, with no carbohydrates detected. Microbial tests over four weeks confirmed the absence of *E. coli*, yeast, and mould, with total microbial activity ceasing by the four week due to acidic conditions. Overall, the pickling method proved effective in enhancing shelf life and meeting production objectives. Further research is recommended to improve product quality, shelf stability, and safety.

Conflict of interest

The authors declare no conflict of interest.

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